Meta-Analysis of Rat Lung Tumors from Lifetime Inhalation of Diesel Exhaust

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Estimating the carcinogenic potential of exposure to diesel-engine exhaust particulates (DEPs) is problematic. In rats, high concentrations of DEPs (> 1,000 μg/m^3) inhaled over a lifetime result in excess lung tumors. However, data for rats exposed to DEP at concentrations not associated with lung overload are consistent with no tumorigenic effect. Individual rat studies have only a limited number of exposure groups; therefore, we combined the tumor data from eight chronic inhalation studies in a meta-analysis. Statistical analysis identified a threshold of response between 200 and 600 μg/m^3 average continuous lifetime exposure, consistent with biological-effect thresholds reported by other investigators. Our exposure–response analysis of all rats with < 600 μg/m^3 average continuous lifetime exposure found no tumorigenic effect of DEP in these rats. When we evaluated all rat studies, accounted for a threshold and for inhomogeneity between experiments, and expressed the results in terms of human unit risk (UR), we found a negative maximum-likelihood human UR of -32×10^{-6} per microgram per cubic meter (μg/m^3), but this was not statistically significantly different from zero. Extrapolating the rat upper 95th percentile confidence limit to humans gave an upper-bound human UR of 9.3×10^{-6} per μg/m^3]. This upper-bound human UR, derived from all of the data points (including 1,087 animals below the estimated threshold and 1,433 in the control groups), falls entirely below the range of estimates derived from lung-overloaded rats or from epidemiology of railroad workers. Our meta-analysis of the low-exposure data in rats does not support a lung cancer risk for DEP exposure at nonoverload conditions. Average ambient concentrations of DEP (0–3 μg/m^3) are < 1% of the concentration associated here with a threshold of tumor response in the rat bioassay. *Key words*: air pollution, exposure response, inhalation toxicology, lung cancer, lung overload, multistage model, risk assessment, threshold of response, unit risk. *Environ Health Perspect* 107:693–699 (1999). [Online 23 July 1999]
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Airborne diesel-engine exhaust particles (DEPs) are small in size and can be inhaled and retained in the respiratory tract. Furthermore, chemicals with mutagenic activity can be extracted from DEP with organic solvents, which caused concern as to potential lung cancer risk to humans (*1*). The results of both occupational epidemiology and studies of rats exposed to high concentrations of DEP (> 1,000 μg/m^3) have been used in efforts to quantitatively assess DEP carcinogenicity. In the case of DEP, fundamental drawbacks limit the usefulness of either of these data sources for quantitative risk assessment, particularly for the low average ambient concentrations (0–3 μg/m^3) to which the general population is exposed (*2*).

Epidemiology

Epidemiologic studies of occupational groups have repeatedly reported weak associations with lung cancer [reviewed by Bhatia et al. (*3*)], but the causal role for DEP in these associations is in doubt (*4,5*). Available epidemiologic studies lack concurrent DEP exposure data, and surrogate measures of DEP exposure do not yield a reliably positive dose response (*6,7*). Control for smoking in the epidemiologic studies has been either absent or inadequate (*4,8–13*). The odds ratios reported across occupations with markedly different potentials for DEP exposure (*3*) are unexpectedly similar, and a recent study of diesel-exposed miners (one of the occupations where DEP exposure is potentially the greatest) reported a deficit in lung cancers (*14*). Thus, the use of diesel-exhaust occupational epidemiology for quantitative risk assessment is problematic.

Laboratory Animal Studies

Among laboratory animals (rats, mice, and hamsters) tested by lifetime inhalation exposure to high concentrations of DEP, only rats develop lung tumors (*15*). Chronic inhalation of DEP at high concentrations can lead to lung overload, where retention of particles in the lungs leads to a decrease in clearance rates, and where there is a progressive increase in the quantity of lung-retained particles (*14*). In rats, lung overload leads to exaggerated lung inflammation and an elevation of lung tumors, a sequence of responses not seen in other species. Lung tumors in high-dose rats are believed to arise from the sequellae of the rat-lung overload and are not specific to DEP [e.g., excess lung tumors have been found in rats that inhaled carbon black particles, which are similar to DEP but have far less extractable organics (*16*)]. Therefore, the lung tumor response in heavily exposed laboratory rats is not believed to be relevant to humans (*8,17–22*), and the high-dose rats are not a reliable basis for DEP quantitative risk assessment.

Mechanistic Evidence for a Threshold Level in the Rat Lung-Tumor Response

Under conditions of lung overload, rat lungs develop tumors in response to many inhaled insoluble particles, although at low levels of exposure, when rat lung clearance is not impaired, the animals do not exhibit exaggerated lung inflammation or develop tumors (*15,19*). Driscoll et al. (*23–25*) examined the inflammatory and mutagenic responses of rats exposed to various concentrations of α-quartz, carbon black, or titanium dioxide. In rats that inhaled 1, 7, or 50 mg/m^3 carbon black for 13 weeks, the authors observed increases in bronchoalveolar lavage fluid neutrophils and mutations in lung epithelial cells at the two higher exposures but not at 1 mg/m^3 (*23*). When α-quartz, carbon black, or titanium dioxide was instilled into rat lungs, mutation data correlated with inflammatory responses. Furthermore, inflammatory cells taken from the lungs of rats exposed to particles could produce mutations in lung epithelial cells *ex vivo*; the particles by themselves were not effective in producing mutations in cultured lung epithelial cells. Thus, mutation effects in rats were dose dependent, and for particle concentrations that did not elicit marked inflammation, mutations in epithelial cells did not occur.

In particle-inhalation studies, a commonly used exposure metric is calculated by multiplying the exposure chamber concentration by the total number of hours per

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week that animals were in the exposure chamber. For example, rats exposed to 3.5 mg/m^3 for 7 hr/day, 5 days/week have an exposure rate of 122.5 (mg-hr)/m^3 per week. Investigators have consistently noted that, for exposure to a wide range of low toxicity particles [above approximately 100 (mg-hr)/m^3 per week], the lungs of exposed rats exhibit responses that are mechanistically related to tumorigenesis. For example, Stöber and Mauderly (*26*) described the onset of clearance impairment at approximately 100 (mg-hr)/m^3 per week. Similarly, Watson and Valberg (*27*) summarized studies showing a dramatic onset of neutrophil influx at approximately 100 (mg-hr)/m^3 per week. Watson and Green (*28*) also evaluated the occurrence of epithelial-cell hyperplasia in particle-exposed rats and concluded that at exposures < 70 (mg-hr)/m^3 per week, hyperplasia was minimal or absent. Also, when inhaled-particle lung burden is expressed in a particle surface area metric, a common threshold in lung tumor response is observed at a dose corresponding to approximately 100 (mg-hr)/m^3 per week [diesel particulate has a surface area of approximately 20 m^2/g (*24*)]. In terms of continuous rat lifetime (30-month) average exposure, 100 (mg-hr)/m^3 per week for 30 months would translate into 595 µg/m^3 {i.e., [100,000 (µg-hr)/m^3 per week]/[168 hr/week]}.

Meta-Analysis of Low-Exposure Rats

For many low-toxicity particles, the overloaded rat lung responds with unique cellular responses, and nonthreshold extrapolation from high concentration results to low concentrations is not valid. However, long-term bioassays are probably the most useful laboratory models for predicting human response. Thus, we need to focus on the low-DEP-exposure rats that provide data in the range relevant to human risk assessment. Previous attempts to use the rat lung tumor data in extrapolations to human risk have focused on individual studies where a finding of no detectable response in one or two low-dose animal groups was not considered sufficient to establish a threshold [reviewed by the California Environmental Protection Agency (*2*), the World Health Organization (*11*), Valberg and Watson (*29*), and Stayner et al. (*30*)]. Because > 4,500 rats have been tested in long-term DEP experiments, a meta-analysis of all available rat data as a whole permits a robust statistical test for the existence of a threshold and allows a reliable estimate of tumor response in the low-exposure range.

In view of the mechanistic evidence for a response threshold at approximately 100 (mg-hr)/m^3 per week, we directly evaluated the rat data for statistical evidence of a threshold in lung tumor response between high and low exposure concentrations. We combined eight lifetime studies of DEP-exposed rats (4,628 animals) and tested whether the rat lung tumor data are consistent with the absence of a threshold. We examined the data below the threshold for heterogeneity with respect to zero-exposure responses and slope of the exposure–response curve, and appropriately pooled the data on rat lung tumors from continuous lifetime average DEP inhalation at concentrations 0–600 µg/m^3 (equivalent to 0–100 (mg-hr)/m^3 per week). We used a multistage

Table 1. Summary of eight studies in which rats were exposed to diesel exhaust.

Study reference (exposure duration)	Diesel exhaust particle concentration during exposure (mg/m^3)	30-month average continuous concentration (µg/m^3)	Number of animals with lung tumors (benign or malignant) Without squamous cysts[a]	With squamous cysts[b]	Number of animals examined
Male and female F344 rats					
Mauderly et al. (*35*)	0	0.0	2	2 (0 f + 2 m)	113 f + 117 m
(35 hr/week, 30 months)	0.35	73	3	3 (2 f + 1 m)	111 f + 112 m
	3.5	730	6 (2 f + 4 m)	8 (4 f + 4 m)	114 f + 108 m
	7.08	1,480	18 (9 f + 9 m)	29 (16 f + 13 m)	108 f + 119 m
Nikula et al. (*16*)	0	0.0	3	3 (0 f + 3 m)	105 f + 109 m
(80 hr/week, 24 months)	2.44	930	13 (8 f + 5 m)	17 (11 f + 6 m)	105 f + 105 m
	6.33	2,400	38 (29 f + 9 m)	55 (42 f + 13 m)	106 f + 106 m
Ishinishi et al. (*34*),	0	0.0	4 (2 f + 2 m)[c]		59 f + 64 m
Takaki et al. (*38*)	0.11	63	3 (2 f + 1 m)[c]		59 f + 64 m
(96 hr/week, 30 months)	0.41	230	1 (0 f + 1 m)[c]		61 f + 64 m
	1.18	670	5 (2 f + 3 m)[c]		59 f + 64 m
	2.32	1,330	3 (1 f + 2 m)[c]		60 f + 64 m
Ishinishi et al. (*34*),	0	0.0	1 (1 f + 0 m)[c]		59 f + 64 m
Takaki et al. (*38*)	0.46	260	1 (0 f + 1 m)[c]		59 f + 64 m
(96 hr/week, 30 months)	0.96	550	0[c]		61 f + 64 m
	1.84	1,100	4 (1 f + 3 m)[c]		59 f + 64 m
	3.72	2,100	8 (3 f + 5 m)[c]		60 f + 64 m
Brightwell et al. (*36*)	0	0.0	4 (1 f + 3 m)[c]		124 f + 126 m
(80 hr/week, 24 months)	0.7	270	1 (0 f + 1 m)[c]		56 f + 56 m
	2.2	840	14 (11 f + 3 m)[c]		56 f + 56 m
	6.6	2,500	55 (39 f + 16 m)[c]		56 f + 55 m
Lewis et al. (*37*)	0	0.0	6 (f + m)[c,d]		180 (f + m)
(35 hr/week, 24 months)	1.95	325	8 (f + m)[c,d]		183 (f + m)
Female Wistar rats					
Heinrich et al. (*33*)	0	0.0	0	0	96
(95 hr/week, 35 months)	4.24	2,800	9	17	95
Heinrich et al. (*39*)	0	0.0	1	1	217
(90 hr/week, 24 months)	0.84	360	0	0	198
	2.50	1,100	4	11	200
	6.98	3,000	9	22	100

Abbreviations: f, female; m, male.
[a]Rats with malignant or benign neoplasms; squamous cysts (lesions identified by the original authors) not included. [b]Rats with malignant or benign neoplasms; squamous cysts (lesions identified by the original authors) included. [c]Squamous cysts not identified by original authors. [d]Study data did not distinguish males and females.

model to determine both maximum likelihood estimates (MLE) and upper confidence limit (UCL) estimates for the exposure–response slope. We tested the sensitivity of the low-dose exposure–response estimates to the assumed threshold by using a consistent dose–response model that included a threshold. We extrapolated our results to human exposure to yield MLE and upper-bound values for DEP unit risk (UR). Finally, we compared our results to those derived from other methodologies.

Methodology and Results

Selection of data. For our meta-analysis, we used studies in which rats were exposed to whole DEP for 24 months or more, where the total number of animals examined per exposure level was > 90, and where the end points examined included lung tumors. We used data on examined animals as reported by the original study authors, but we excluded animals sacrificed prior to the completion of 12 months of DEP exposure.

In the classification of rat lung tumors, there has been some debate about the status of squamous keratin cysts, which have been variously called "benign cystic keratinizing squamous cell tumors" and "cystic keratinizing epitheliomas" (*31,32*). Because the classification of these lesions occurred subsequent to the original pathology, we performed our analysis two ways. One analysis excluded squamous cysts from those studies where they were explicitly identified as such, and our other analysis included squamous cysts with lung tumors. Because squamous cysts occur only at elevated exposure concentrations, their exclusion or inclusion has no effect on the exposure–response curve below threshold.

Using these criteria, we identified eight chronic inhalation studies (*16,33–39*). Some studies include results from animals sacrificed at interim periods (*36,37*). The exposure concentrations and study results are summarized in Table 1, and Figure 1 illustrates data on the proportion of animals (males and females combined) with a lung tumor as a function of exposure to DEP, using the metric described below. Table 1 lists six studies on male and female F344 rats and two on female Wistar rats. The lung tumor column of Table 1 shows numbers of rats with malignant or benign neoplasms, either including or excluding lesions identified by the original authors as squamous cysts.

Because male and female rats may respond differently to DEP, experiments on males and females were treated, wherever possible, as distinct for analytical purposes; one study (*37*) did not distinguish male and female rats. With the male–female separation, Table 1 lists thirteen distinct exposure–response experiments in which rats were exposed to DEP at various concentrations with various exposure regimes. However, analyses combining male and female results within studies came to essentially identical conclusions.

For our DEP exposure metric, we standardized the different DEP exposure patterns used in the rat studies to a metric that is proportional to cumulative exposure. Our metric is the 30-month average continuous exposure expressed in units of micrograms per cubic meter. For example, 0.7 mg/m^3, 80 hr/week, for 24 months = [(700 µg/m^3) (80 hr/week) (24)]/[(168 hr/week) (30)] = 270 µg/m^3, continuous 30-month exposure.

Data for all exposure concentrations (males and females combined) are plotted on Figure 1, but we also selected low-exposure groups for closer examination. In the 13 experiments shown in Table 1, 10 (*35–39*), provided 14 low-exposure groups where the lifetime DEP exposure level was below approximately 100 (mg-hr)/m^3 per week (< 600 µg/m^3, equivalent continuous lifetime exposure), along with 10 corresponding control groups. These 24 groups provided 1,212 DEP-exposed rats and 1,123 clean-air-exposed (control) rats. For these groups, Figure 2 illustrates the combined male and female data showing percent increases or decreases of lung tumors in comparison to clean-air controls as a function of DEP lifetime-exposure concentration from 0–600 µg/m^3.

Statistical examination of the data for a threshold in the lung tumor response. To test for the existence of a threshold in tumor response, we used all 13 experiments given in Table 1. The metric of DEP exposure is given in Table 1, column 3. Using standard maximum likelihood methods, we fitted all of the lung tumor data shown in Table 1 with a flexible exposure–response model containing a nonnegative (but possibly zero) threshold concentration, d_t, and of the form:

$$p = 1 - \exp(-a_0) \text{ for } d \le d_t$$
$$= 1 - \exp\{-[a_0 + a_1 (d - d_t) + a_2 (d - d)^2 + a_3 (d - d_t)^3 + a_4 (d - d_t)^4]\} \text{ for } d \ge d_t \quad [1]$$

where p is the probability for any lung tumor, d is the exposure concentration, and the $a_i \ge 0$ are the parameters of this exposure–response model. MLEs for the threshold for each experiment and for all experiments simultaneously were evaluated, tested for equality, and tested for deviation from zero as described below.

This model was chosen to be consistent with the standard multistage model used by the U.S. Environmental Protection Agency (EPA) (*40*). This exposure–response equation has a constant probability of tumor below the threshold. We subsequently model the below-threshold response with an exposure–response equation that allows for increasing or decreasing probability of tumors. We could do both together and later we do so to test for sensitivity to the threshold value. However, when testing for evidence of a threshold, it is more conservative

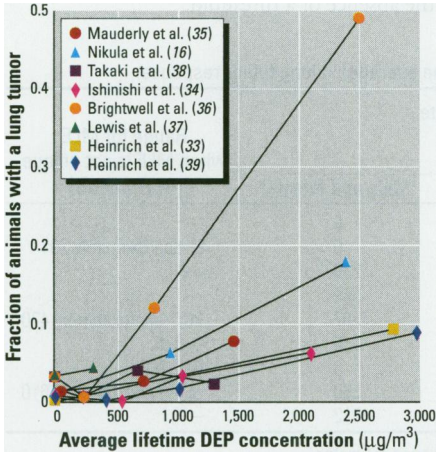

Figure 1. Rat lung tumor outcome (fraction of animals with a lung tumor) as a function of lifetime inhaled DEP concentration (see text for definition of this dose metric). DEP, diesel exhaust particle. Data from all eight studies listed in Table 1 (using the data for all tumors, but not including squamous cysts).

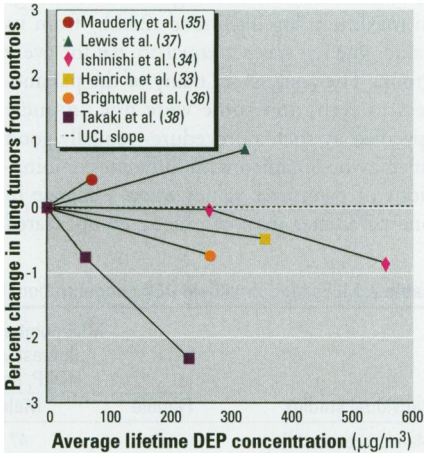

Figure 2. Rat lung tumor outcome (percent change from control) as a function of lifetime inhaled DEP concentration. Abbreviations: DEP, diesel exhaust particle; UCL UR, 95th percentile upper confidence limit unit risk. Data from the eight low-exposure groups (< 600 µg/m^3 average continuous lifetime exposure) taken from the studies listed. The visual appearance of the data suggests an absence of excess lung tumors from DEP within the 0–600 µg/m^3 concentration range. Analysis of the data from low-exposure groups simultaneously (Table 1) using a multistage model (Equation 3) modified to allow negative slope values yields a negative (not statistically significant) linear coefficient (-35×10^{-6} per µg/m^3). The UCL UR, 0.3×10^{-6} per µg/m^3, is also shown.

(more likely to reject the presence of a threshold) to ignore any evidence of decreasing probability of tumors.

Because there were a maximum of five exposure groups in any study, five parameters (a_0–a_4) were used for each study, although the analysis set many of the parameters to zero (i.e., not all the parameters were needed). We assumed that the observed tumor response was binomial with a probability given by the exposure–response model at the experimental exposure; the same procedure is used by the EPA for fitting the multistage model (*40*). The model parameters were adjusted by comparing the model prediction for tumor probability, p, to the actual number of tumor-bearing animals, r, in each exposure group of total size, n. That is, for each exposure group in which r of n animals were observed to have a tumor, the log likelihood was computed as

$$r \ln(p) + (n - r) \ln(1 - p) \qquad [2]$$

where p is obtained from the exposure–response model at the relevant exposure concentration, and the total log likelihood is the sum of such terms for each exposure group of every exposure–response result.

All 13 experiments were fitted with exposure–response equations, allowing different parameters and threshold concentrations for each set of results. In all cases the fit was adequate, i.e., the assumed model was not rejected by a test using twice the change in maximum log likelihood (2ΔLL) from its value when p was set equal to r/n for every group. The analysis set many of the parameters to zero, and some were not uniquely specified by such a procedure—equally good fits may be obtained with different combinations of parameter values when a change in one parameter may be exactly compensated by a change in others. However, adopting this approach allows the greatest freedom in fitting the results and minimizes any constraints caused by the selection of a particular exposure–response shape. Because only the threshold concentration d_t was of interest in this phase of our analysis, no attempt was made to determine whether any parameters other than d_t were homogeneous across experiments.

The MLE for the threshold concentration in each experiment individually and the MLE for all experiments together are shown in Table 2 for the case where squamous cysts are included. Where there is a range of possible values for the MLE, the point estimate shown is the lowest maximum likelihood (ML) value. When all experiments were assumed to have the same threshold (the homogeneity assumption), 2ΔLL = 1.51, which is not significant when tested using the standard likelihood approximation of treating 2ΔLL as a χ^2 variate. Thus, the experiments are all statistically consistent with a single threshold of 512 μg/m^3, in good agreement with histopathology data. However, the set of experiments is not consistent with a threshold of zero. Setting the threshold equal to zero gives 2ΔLL = 5.73 (compared with the ML for a single threshold), with p = 0.017 (one-sided). Figure 3 shows the likelihood profile for the threshold concentration for the analysis with squamous cysts included (green line) or excluded (blue line).

The combined data are all statistically consistent with a single non-zero threshold near 500 μg/m^3 in the exposure–response curve; the MLE was 512 μg/m^3 with squamous cysts included and 478 μg/m^3 with squamous cysts excluded. This threshold is statistically different from zero—the exposure–response curves are not consistent with the absence of a threshold.

To evaluate the exposure–response curve below threshold using the maximum amount of low-exposure data, we selected a lifetime-concentration cutoff of 600 μg/m^3 [~ 100 (mg-hr)/m^3 per week]. This concentration agrees with the biological evidence discussed earlier for a threshold in lung clearance impairment and in the onset of lung inflammation in rats. Moreover, this value is statistically consistent with the non-zero threshold derived above. Selecting a cutoff that is too high will bias best estimates of effect upward (by including exposure groups above the threshold, where different mechanisms may act), whereas selecting too low a value will bias upper confidence bounds upward (by reducing the effective numbers of animals included). We also provide a rigorous analysis that avoids the necessity of selecting a single value for the threshold.

Derivation of an MLE and a UCL to the exposure–response slope. To calculate the lung tumor effect of low exposures of DEP in rats, we combined data from studies where rats had < 600 μg/m^3 continuous lifetime concentration exposure. Because no squamous cysts were reported for exposures below this concentration, the analysis does not depend on whether such lesions are included or excluded. Below this cutoff, Table 1 shows a subset of six studies, including 10 control groups (zero exposure) and 14 DEP-exposed groups (exposures ranging from 63 to 550 μg/m^3). Including control

Table 2. MLEs for a threshold DEP concentration (lifetime average) in lung tumor response.

Individual studies	MLE point estimate, lowest value (DEP, μg/m^3) Female	Male	Male and female[a]	Range for MLE when MLE is not unique (DEP, μg/m^3)[b]
Mauderly et al. (*35*)	0	47	0	—[c]
Nikula et al. (*16*)	0	0	0	0–approximately 291
Takaki et al. (*38*)	0	234	234	—[c]
Ishinishi et al. (*34*)	549	549	549	—[c]
Brightwell et al. (*36*)	267	267	267	267–approximately 470
Lewis et al. (*37*)	–	–	0	0–325
Heinrich et al. (*33*)	0	–	0	0–2,797
Heinrich et al. (*39*)	360	–	360	360–approximately 610
All studies simultaneously	–	–	512	—[c]

Abbreviations: DEP, diesel exhaust particle; MLE, maximum likelihood estimates.
[a]Shows the threshold assuming it to be identical for the female and male rats in the second and third columns. [b]Range of parameters to obtain equal likelihood. There are sufficient parameters available in the dose–response equation that the maximum likelihood value for any one of them may not be unique for individual experiments with only a few doses. In particular, several of the individual experiments may be fitted with equal likelihood for any threshold level within a range of values; for each threshold value within the range, the other parameters may be adjusted to obtain the same value for the likelihood. The exact range is not of particular interest; therefore, the upper ends have only been approximately evaluated in three cases, whereas for two other cases the exact upper end is easy to compute. [c]Where no range is given, the MLE is unique.

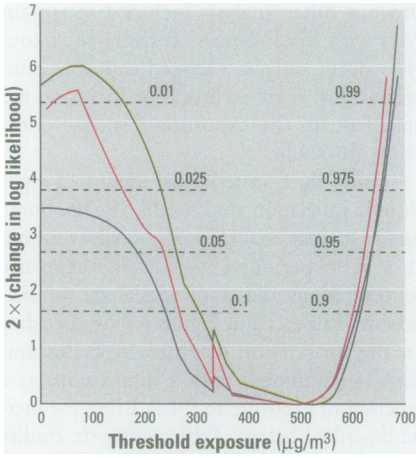

Figure 3. Likelihood profiles for the threshold concentration. Twice the change in maximum loglikelihood as a function of threshold concentration in the exposure-response equation. The green line shows Equation 1 with squamous cysts included; the blue line shows Equation 1 with squamous cysts excluded; and the magenta line shows Equation 1 above threshold and Equation 3 below threshold, with squamous cysts included. The dashed lines and values in the figure represent the lower and upper approximate confidence levels. The slight kink in the likelihood profile at 325 μg/m^3 is real, and is due to the Lewis et al. study (*37*), with only one non-zero exposure at 325 μg/m^3.

groups from other studies would not contribute any information because the analysis independently estimates the background rate for each experiment. Exposures below equivalent-lifetime-concentrations of 600 μg/m^3 are not characterized by an adverse cellular response or a lung-tumor response. Thus, our *a priori* hypothesis is that there could be either a low-exposure tumorigenic effect or a low-exposure anti-tumorigenic effect (*41*).

To estimate the potential effect of DEP at low exposures with as few assumptions as possible, we fitted the exposure–response model:

$$p = 1 - \exp\left[-a_0 \exp\left(\frac{a_1 d}{a_0}\right)\right] \quad [3]$$

In this equation, a_0 measures background probability of lung tumors, and a_1 is a measure of the slope of the dose–response curve. This functional form was chosen so that at low doses a_0 and a_1 have effectively the same meanings as in the standard EPA multistage model, except that a_1 can be negative if there is some antitumorigenic effect; otherwise, no biological meaning is implied. The functional form is linear at low doses and does not lead to negative probabilities at higher doses. If $a_0 = 0$, then a_1 cannot be negative; if $a_1 > 0$, then a_0 must be positive. Because > 90% of chemicals tested in rodent cancer bioassays produce a significant tumor decrease in at least one site in one or more tested groups (*42*), a hypothesis incorporating the possibility of low-dose antitumorigenic effects is warranted, in contrast to the presumption adopted by the EPA. The experimental results from the low-exposure groups were fit by maximum likelihood methods identical to those used in the standard EPA multistage model-fitting approach (*40*).

With this approach, we tested the null hypothesis $a_1 = 0$ (no effect) versus alternate hypotheses that DEP increases or reduces lung cancer risk in rats at these low exposures. We also estimated a UCL on the coefficient a_1.

We fitted Equation 3 independently to the low-exposure (< 600 μg/m^3) groups in the 10 experiments on Table 1 that had such groups. We compared the individual results to results obtained by fitting the low-exposure groups simultaneously. We found that the UR a_1 is homogeneous across experiments [2ΔLL = 7.89, p = 0.54 on 9 degrees of freedom (*df*)], with an MLE of -35×10^{-6} per μg/m^3.

With a homogeneous a_1, the background lung tumor rates for the experiments are significantly inhomogeneous (2ΔLL = 24.7, p = 0.0033 on 9 *df*); therefore, it would be inappropriate to perform any analysis that lumps the zero-exposure groups together.

The MLE UR a_1 (rats) is not statistically significantly different from zero (2ΔLL = 2.61, p = 0.11, two-sided). Thus, although the MLE suggests that the effect of low exposures of DEP is slightly antitumorigenic, one cannot reject the null hypothesis of no effect below the threshold concentration. The 95% UCL on UR a_1 (rats) obtained by this approach is 0.3×10^{-6} per μg/m^3 obtained by the usual profile likelihood method (*40*). This UCL rat UR is illustrated relative to the low-exposure data in Figure 2.

The usual EPA procedure for the multistage model is to constrain a_1 to be nonnegative, requiring the (flawed) *a priori* assumption that the treatment can only increase tumor risk. If we follow an exact analog of the EPA procedure but do not require equal values for a_0 in each experiment, the MLE for a_1 is zero and the UCL is 6.3×10^{-6} per μg/m^3.

In summary, our statistical analyses of low-exposure rat data suggest zero tumor response for DEP at continuous lifetime exposure concentrations below 600 μg/m^3.

A rigorous treatment for threshold selection. Selecting a threshold value and evaluating only experimental results below that threshold does not allow for the effect of uncertainty in threshold selection. To rigorously account for such uncertainty, we evaluated all the data using a combined exposure–response curve that has the form of Equation 1 above the threshold and Equation 3 below the threshold (with parameter a_0 in Equation 1 selected to join these equations at the threshold concentration, and with a constraint that the slope of the exposure–response curve does not decrease as the exposure increases across the threshold). With such an exposure–response curve and including lesions reported as squamous cysts, the MLE for the threshold is 478 μg/m^3, with a 95% confidence interval (CI), 155–624 μg/m^3. The MLE for a_1 in Equation 3 (the low-dose slope) is -15.5×10^{-6} per μg/m^3 and the upper 95% confidence limit is 4.5×10^{-6} per μg/m^3.

The likelihood profile for the threshold using this combined dose–response curve is shown as the magenta line in Figure 3. Approximate confidence limits obtained from the likelihood profile (cysts included) are as tabulated in Table 3.

Scaling to human UR. The rat UR value can be converted to a human UR by assuming that the appropriate measure of equivalent dose is mass of lung-deposited DEP particles per unit body surface area per day (*29*). Because metabolic rates scale more closely in proportion to body surface area than body weight, body surface area is generally used to normalize dose in cases where metabolism may play a part in the final outcome.

Table 3. Confidence intervals on the threshold diesel exhaust particle exposure.

Interval (%)	Lower (μg/m^3)	Upper (μg/m^3)
80	262	585
90	233	606
95	155	624
98	81	645

Three factors enter into this conversion. First, humans breathe more per day than rats (20 m^3/day vs. 0.35 m^3/day), giving a multiplicative factor of 20/0.35, or 57.14. Second, humans have more body surface area than rats (surface area is assumed proportional to body mass to the 2/3 power), giving a multiplicative factor of $(0.3 \text{ kg}/70 \text{ kg})^{2/3}$, or 1/37.9. Third, deposition efficiency in lung alveoli for inhaled DEP particles is greater in humans (0.15) than in rats (0.11) (*43*), giving a multiplicative factor equal to the ratio of deposition efficiencies (0.15/0.11), or 1.364. Overall, the human UR is obtained by multiplying the rat UR by $57.14 \times (1/37.9) \times 1.364$, or 2.06. If the rat to human extrapolation is unbiased, the MLE UR in humans is -32×10^{-6} per μg/m^3.

Application of the same scaling factor of 2.06 to the UCL UR for the rat gives an upper-bound UR in humans of 9.3×10^{-6} per μg/m^3.

Likewise, a threshold concentration of 478 μg/m^3 (CI, 155–624 μg/m^3) in rats extrapolates to an equivalent human lifetime exposure threshold concentration of 230 μg/m^3 (plausible range 75–300 μg/m^3). However, because humans are not known to exhibit the lung-overload and associated sequellae observed in rats, these estimates should be interpreted as a lower bound on the likely higher human threshold concentration.

If, instead of body surface area scaling, we use a body weight scaling factor (1/233), the predicted human UR is reduced by a factor of 6.1 relative to the UR obtained from body surface area scaling. The human MLE UR using body weight scaling would be -5.2×10^{-6} per μg/m^3; the upper-bound human UR would be 1.5×10^{-6} per μg/m^3, and the human lifetime exposure threshold concentration would be 1,400 μg/m^3.

Scaling to lung (alveolar) surface area, which varies as the 3/4 power of body weight, would give results intermediate between those for the above two methods for extrapolation to humans.

Discussion

Meta-analysis of epidemiologic studies for improving statistical precision is common. We are unaware of any previous meta-analysis of the laboratory data on diesel-exposed rats. Although the rat studies were conducted in different laboratories with somewhat different protocols, the DEP exposures, the particle-size parameters, the test conditions, and tumor outcome assessment were far more

homogeneous than possible in separate epidemiology studies. Meta-analysis of the rat data provided an unequivocal demonstration of a threshold in DEP exposure response, and incorporating this threshold provided an upper limit on DEP lung cancer risk in the test range of concentrations.

The meta-analytic result for low-exposure rats can be compared and contrasted with results from other approaches to deriving DEP UR values. These approaches include using rat results across all DEP exposure concentrations without taking into account the possibility of a threshold, and using a limited number of the epidemiologic studies with attendant assumptions about historical exposure. We also compared our results to those obtained assuming no threshold, but by applying EPA methodology for removing highest-dose groups when the standard EPA dose–response function does not adequately fit the all the data.

Comparison with high-exposure estimates of DEP UR. The World Health Organization (WHO) used a different fitting procedure (*11*), included the high-exposure rats, and included squamous cysts as tumors. Using the approach we have described but applying to the low-dose data an exposure–response curve of the form

$$p = 1 - \exp[-(a_0 + a_1 d)],$$

and with the parameters restricted to be positive, we find that the MLE for the rat UR a_1 is zero and its UCL is 7.2×10^{-6} per $\mu g/m^3$ when different values for a_0 are allowed for the different experiments. Using a dose–response curve that has this form at low doses, the WHO (*11*) used high dose results in rats to estimate an upper confidence limit for human UR (geometric mean of four studies from four separate fits) at 34×10^{-6} per $\mu g/m^3$ (corresponding to a rat UR of approximately 16.5×10^{-6} per $\mu g/m^3$). The dose metric used by the WHO was lung particle burden per unit lung surface. Testing the WHO mean value for consistency (using likelihood ratios) with our meta-analysis gives a probability of just 0.0013 (one-sided). The WHO result, derived from high-dose rats, is biased upward and is markedly inconsistent with our results based on low-exposure rats only.

Comparison with epidemiology estimates of DEP UR. Using data from epidemiology of DEP-exposed workers, the California Air Resources Board (*2*) obtained a human UR estimate of 300×10^{-6} per $\mu g/m^3$. Back-extrapolation of this result to a rat UR as described in the text gives approximately 140×10^{-6} per $\mu g/m^3$. This value is dramatically inconsistent with the results from low-exposure rats ($p < 10^{-10}$ using a likelihood ratio test).

Comparison with EPA methodology of recursive removal of high-exposure groups. We consider the analyses we used (threshold identification plus fitting of low-exposure groups or fitting of a complete exposure–response curve with a threshold concentration) to be the best available for the data described by Table 1. However, it is of some interest to evaluate the totality of results shown in Table 1 exactly according to EPA methodology, presuming that no mechanistic information about a threshold is available.

A direct comparison among the control groups in each study shows no significant inhomogeneity ($\chi^2 = 16.4$ on 12 *df*, $p = 0.17$), suggesting that all the studies can be considered as a single large study with males and females combined at each exposure. In this scenario, the EPA methodology (*40*) can be applied to the resulting set of 22 exposure groups (the eight control groups being treated as a single control group in which 21 of 1,443 animals had lung tumors). EPA methodology calls for maximum likelihood fitting of a dose–response function of the form

$$p = 1 - \exp[-(a_0 + a_1 d + a_2 d^2 + \ldots + a_{k-1} d^{k-1})]$$

at exposure or dose d, where p is the lifetime probability of tumor and the a_i are parameters that are all constrained to be positive. The number of parameters, k, must be chosen as equal to the number of dose groups. If the dose–response function does not adequately fit all the data according to a χ^2 test described by Anderson et al. (*40*), the highest dose groups should be recursively removed from the analysis until the test is satisfied.

Strict application of this procedure to the 22 exposure groups of Table 1 results in 10 of the exposure groups being recursively dropped from the analysis because of failure to fit the data, which leaves only those exposure groups with average exposure concentrations ≤ 840 $\mu g/m^3$. With only these exposure groups included, the MLE for the UR, a_1, is then zero, with UCL equal to 18.9×10^{-6} per $\mu g/m^3$ (including the squamous cysts). This value is higher than that we derived principally because of the failure to account for the threshold and because of failure to account for the inhomogeneity with respect to a_0 and a_1. Our method, which accounts for both, is thus preferable. In summary, rigorous application of EPA methodology even in the absence of an assumption about threshold gives an MLE estimate of zero for the slope, but with a considerably higher UCL because of failure to account for either the threshold or the inhomogeneity among the various studies. Overall, the method we chose is superior to a blind application of a procedural recipe that does not consider the data being modeled.

In our extrapolation to humans, we did not specifically allow for species differences that are known to exist in the clearance rate for lung-deposited particles (*44*). However, the factor of 2.06, which we used to relate (i.e., reduce) continuous lifetime rat exposure to DEP to yield an equivalent human continuous lifetime human exposure, is conservative. For example, in the WHO Environmental Health Criteria document on diesel exhaust (*11*), the deposition-clearance dosimetric model of Yu and Yoon (*45*) was used to scale rat to human exposure. This model specifically allows for differences between rates of lung clearance for rats versus humans, and the factor obtained to relate (i.e., reduce) continuous rat exposure at low concentrations to equivalent human lifetime exposure was 1.66 (*11*). Had we applied this factor instead of the 2.06 value, we would have predicted a lower UR and a higher threshold concentration for humans.

Conclusions

Our meta-analysis of data from laboratory rats supports the conclusion that the tumor responses observed at high levels of DEP exposure do not occur at low exposures. Both the statistical modeling of lung-tumor data we presented, as well as studies by other investigators on the cellular effects associated mechanistically with rat lung tumors, indicate a threshold of response in the range of 160–600 $\mu g/m^3$ continuous lifetime concentration.

The low-exposure rats provide a quantitative best estimate of no DEP tumorigenesis below a threshold of approximately 480 $\mu g/m^3$. Using all of the available data on rats, accounting for the threshold and for the inhomogeneity between experiments, and extrapolating the result to humans, we calculated a negative MLE UR of -32×10^{-6} per $\mu g/m^3$, which is not statistically significantly different from zero. The upper-bound UR is 9.3×10^{-6} per $\mu g/m^3$.

The null result in two strains of rats at low exposure, plus the null results in long-term bioassays of DEP-exposed mice (*46*) and DEP-exposed hamsters (*33,36*) give strong support from the laboratory animal models for a lack of DEP carcinogenicity in humans below lifetime exposure threshold concentrations at least as high as 230 $\mu g/m^3$ (plausible range on this estimate is 75–300 $\mu g/m^3$). A low level of risk is also supported by a comparative potency analysis between DEP and cigarette smoke (*47*).

The UCL bound on UR we calculated from the low-exposure 2-year rat bioassay data exclude ranges of risk calculated by other approaches. The upper-bound UR (9.3×10^{-6} per $\mu g/m^3$) derived from the more than 4,600 animals (including 1,433 controls and 1,087 exposed below the estimated

threshold concentration) falls entirely below the range of URs for humans recently estimated from the high-exposure-level rats [UR = 34.0×10^{-6} per $\mu g/m^3$; (*11*)] or from the occupational epidemiology [UR = 300×10^{-6} per $\mu g/m^3$; (*2*)].

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